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The Possible Ameliorative Effect of Ginseng and Vitamin D on Citalopram Induced Fertility Dysfunction on Male Rats

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Abstract | There is an increasing worry that antidepressant medications negatively affect spermatogenesis, male fertility, and sexual function. This study aimed at investigation of the possible mitigating effects of ginseng and vitamin D against citalopram-induced testicular toxicity and damage in male rats. Thus, 42 adult male rats were randomly, and blindly divided into 7 groups equally: control (G1, received 0.2ml saline/rat/day), citalopram (G2, received 1.8 mg/kg b.wt), ginseng (G3, received 18 mg/kg b.wt), vitamin D (G4, received 0.01mg/kg b.wt), citalopram plus ginseng (G5), citalopram plus vitamin D (G6), citalopram plus ginseng and vitamin D (G7). At the end of treatments, testicular levels of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), malondialdehyde (MDA), serum hormonal and sperm parameters, and histopathology of testes were measured. The obtained results were exposed to one-way analysis of variance followed by *post hoc* Tukey's test to assess the statistical significance of data between different groups. The results revealed that oral treatment with citalopram for 60 days ensued significant (p<0.001) upsurges in the levels of luteinizing hormone (LH), and follicle-stimulating hormone (FSH) in serum with non-significant (p > 0.05) alterations in serum testosterone (TS) level when compared to the control group. Treatments by citalopram plus ginseng, vitamin D, or both caused significant declines in the circulating serum TS (p<0.01), LH (p<0.001), and FSH (p<0.001) levels matching to G2. Citalopram had detrimental effects on male rat fertility, as evidenced by a significant reduction (p<0.0001) in sperm counts and motility and a large rise (p<0.0001) in the percentage of defective sperms. By contrast, different treatments (G 6-7) restored spermatogenesis by significant improvement in the sperms count and sperm morphology when compared with G2. There are regressive histopathological alterations in the testis of citalopram-treated rats and these lesions were alleviated with administration of ginseng, vitamin D alone or in combination. In conclusion, ginseng and vitamin D could reduce oxidative stress, necrotic and apoptotic alterations, and protects against testicular damage caused by citalopram. Therefore, they might act as potential candidates to improve citalopram-induced testicular toxicity and damage in male rats.

Keywords: Citalopram, Ginseng, Vitamin D, Antioxidants

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INTRODUCTION

In both industrialized and developing nations, there has been a notable decrease in fertility rates during the last few decades. Approximately 70 million people worldwide suffer from the prevalent issue of infertility (Aşır *et al*., 2024), 15% of all reproductive-aged couples, and 50% of infertility cases are of male origin (Saleem *et al*., 2020). The most commonly identified risk factors for male infertility include aging, stress, smoking, chemotherapy, occupational toxins, environmental pollution, nutrition, and genetics (Aşır *et al*., 2024). These issues, either separately or in combination, may have an impact on sperm production and contribute to overall male infertility and subfertility (Jung and Schuppe, 2007). Furthermore, around 30% of cases of male infertility are caused by abnormalities in the sperm (Cavallini, 2006). Male infertility is primarily associated with hormonal changes brought on by dietary variables (Lotti and Maggi, 2018). These jeopardy reasons are linked to augmented reactive oxygen species (ROS) production and levels of oxidative stress which can oxidize sperm protein, DNA, and lipids, influence the enzymatic function and lead to significant changes resulting in infertility, damaging the sperm quality, and cell death (Redza-Dutordoir and Averill-Bates, 2016). The first cells in which ROS generation was recognized were spermatozoa (Maghsoumi-Norouzabad *et al*., 2021).

The widely held investigations that is now accessible focuses on selective serotonin reuptake inhibitors (SSRIs), which have been proven to have detrimental effects on reproduction. Examples of SSRIs include citalopram, escitalopram, paroxetine, fluoxetine, fluvoxamine, and sertraline (Kumar *et al*., 2024). Compared to other antidepressants in its class, citalopram is a selective SSRI antidepressant with a more focused and selective pharmacological profile (Bezchlibnyk - Butler *et al*., 2000). Treatments with citalopram decreased libido and arousal and anorgasmia in thirty percent of patients (Lorenz *et al*., 2016). Male rats given citalopram both acutely and chronically have less ejaculation and mounting (de Jong *et al*., 2006). In a recent study, citalopram evoked testicular toxicity and spermatogenesis impairment in mice (Moradi *et al*., 2024).

Phytotherapy is based on the idea that plants possess natural properties that support the body and reduce ailments. For this reason, plants have been utilized as energizing supplements, vitamin supplements, and potential enhancers of male sexual performance (Derbak *et al*., 2023). *Panax ginseng* Meyer (ginseng) of the Araliaceae family has been regarded as one of the best natural herbs for enhancing health. Ginsenosides, a broad class of steroidal saponins, are the main active ingredients in ginseng. They have the capacity to target a variety of tissues and elicit a range of pharmacological effects (Li *et al*., 2014). Additionally, ginseng reversed testicular dysfunction in aged rats (Kopalli *et al*., 2015).

Due to its pleiotropic role-which includes autocrine, paracrine, and endocrine actions on a variety of target organs and systems-vitamin D has been a widely studied substance. It is a fat-soluble vitamin that helps to support bone mineralization and preserve calcium and phosphorus homeostasis (Dusilová-Sulková, 2009). Vitamin D is well-thought-out as an imperative micronutrient with various biological properties (Maghsoumi-Norouzabad *et al*., 2021). Reduced sperm functioning may be the cause of male infertility; low vitamin D levels can impact the number and growth of motile sperm (Aşır *et al*., 2024).

This study aimed at investigation of the possible mitigating effects of ginseng and vitamin D against citalopram-induced testicular toxicity and damage in male rats.

MATERIALS AND METHODS

Animal Ethical Statement

Animal handling, medications, and scarification were carried out following the guidelines for the care of experimental animals and approved by the Research Ethical Committee, Fac. of Vet. Med., Zagazig Univ., Zagazig, Egypt according to the Animal Research Ethics Committee Guide for care and use of laboratory animals (Approval number: ZU-IACUC/2/F/118/2024).

Drugs

- Citalopram tablets [citalopram hydrochloride ($C_{20}H_{22}BrFN_2O$), 20 mg] were gained from Eva Pharmaceutical Com., Egypt.
- Ginseng tablets (Panax Ginseng Extract Tablets - 200mg), natural herbal supplements, were purchased from Ema pharm Co., Egypt.
- Vitamin D tablets (concentrations of 125 mg (5000 IU)] were bought from Eva Pharmaceutical Company, Egypt.

Experimental Animals

Clinically healthy adult male albino rats aged 7 to 9 months and weighing between 150-200 grams were obtained from the Animal House of Fac. of Vet. Med., Zagazig Univ., Egypt. The animals were maintained in metal cages with typical laboratory settings, which included room temperature of around 25°C and aeration. Throughout the experiment, the animals were provided a regular diet consisting of pellets and unlimited access to filtered water *ad libitum*.

Experimental Design

The forty-two rats were divided randomly and blindly into 7 groups, each of 6 rats. Group 1 received 0.2 ml/ day of saline solution orally for 60 days and served as a negative control. Groups 2-4 received citalopram (1.8 mg/kg b.wt), ginseng (18 mg/kg b.wt) and vitamin D (0.01mg/kg b.wt).

Groups 5-7 were given citalopram plus ginseng (G5), vitamin D (G6), ginseng or vitamin D (G7).

All medications were administered orally one time daily for 60 days after being dissolved in regular saline at 0.2 ml containing the above calculated doses according to (Paget and Barnes, 1964). Drug solutions were freshly prepared before administration.

Sampling

On the day 60, rats were euthanized and the blood samples were collected from vena cava, and permitted to be clot at room temp for 10-20 minutes. After that, samples were centrifuged for 15 minutes at 3000 rpm to separate the serum, which was then kept at -20˚C (Hashem *et al*., 2018) until analysis of hormonal parameters.

All of the animals' testicular and epididymal tissues were removed, and they were then cleaned in saline. The epididymis was cleaned and minced in order to determine the morphology and total number of epididymal sperm. Testes was washed in ice cold saline and stored in freezer (–20˚C) for antioxidants measurements. Testicular and seminal vesicles tissues were fixed in 10% formalin for histological studies.

Antioxidants Analysis

The testicular samples stored at –20˚C were thawed and homogenized in 3 milliliters of phosphate buffer (pH 7.4). The homogenate was centrifuged for 30 minutes at 4˚C at 100,800 g. To ascertain the testicular tissue's antioxidant level, supernatant was collected.

The activity of antioxidants like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were estimated in the testicular tissue of experimental animals according to the methods described by (Kakkar *et al*., 1984), (Aebi, 1984) and (Paglia and Valentine, 1967) respectively using Bio Rad SmartSpecTM Plus Spectrophotometer. Lipid peroxidation represented by malondialdehyde (MDA) was assessed in the testicular tissue according to the method of (Mihara and Uchiyama, 1978).

Hormonal Analysis

Serum concentrations of testosterone (TS) (Diametra Co, Italy, with 0.07 ng/ml sensitivity), luteinizinghormone (LH) (Bioassay Technology Laboratory, China, with 0.051 µIU/ml sensitivity) and follicle -stimulating hormone (FSH) (Bioassay Technology Laboratory, China, with 0.12 µIU /ml sensitivity) were measured through enzyme-linked immunosorbent assay using commercial enzyme immunoassay kits (BiocheckInc, USA), according manufacturer's instructions provided with the kit.

Semen Analysis

Epididymal sperm count: The epididymis and vas deferens were minced in 1ml of Phosphate Buffer Saline (PBS) (pH 7.2) to obtain a suspension. The sperm count was counted using the standard hemocytometric method using the improved Neubauer hemocytometer (Deep 1/10 mm, LABART, Germany) as described by (Marianti *et al*., 2020). Briefly, an aliquot from the suspension (up to 0.5) was taken in leukocyte pipette and diluted with PBS up to the mark 11. Prior to the count, the chamber was washed and cleaned. A cover slip was placed on the counting chamber. The sperm suspension was well-mixed and charged in to Neubauer counting chamber and examined under a light microscope with x10 objective lens. The total sperm count in eight squares (except the central erythrocyte area) of 1 mm^2each was determined and multiplied by 5x $10^4$to calculate the number of spermatozoa per milliliter.

Evaluation of sperms motility: On a slide that had been preheated beforehand, sperm from the epididymis filtrate were wet mounted. The percentage of spermatozoa that moved gradually in one direction on a slide per field was observed using a light microscope to count the motile and non-motile sperms. Spermatozoa that exhibited round, pendulous, or retrograde movements were not counted; only those traveling in a single direction were counted (Zemjanis, 1970).

Assessment of sperm morphology: Fresh sperm smears were prepared for morphometric analysis by placing 5 µl of the fresh semen on the clear end of a frosted slide by dragging the drop across the slide. The smears were air-dried before staining. Three semen smears were prepared and stained with Eosin nigrosine stain. The assessment of sperm morphology was qualitative and quantitative and included microscopic evaluation for atypical spermatozoa morphology involving any changes involving the head, neck, mid-piece and tail and the diagnosis of abnormal spermatozoa (Horri *et al*., 2021).

Histopathological Analysis

The formalin preserved specimens collected from rat's testicular and SV tissues were processed in an automated tissue processor. The samples were then cleared in multiple changes of xylene after being dehydrated using a gradient of ethanol concentrations (70–100%). After that, samples were imbedded, blocked out, and impregnated with melted paraffin wax. Hematoxylin and eosin was used to stain 4-to 5-micrometer paraffin sections (Suvarna *et al*., 2018). A veterinary pathologist looked at the pathologic structural abnormalities under a light microscope.

Statistical Analysis

The standard error of the mean (mean ± SEM) was used

to express the results. Version 22 of the SPSS program was used to conduct the statistical analysis (IBM corporation, SPSS Inc., 2010). One-way analysis of variance (ANOVA) followed by *post hoc* Tukey's test was used to assess the statistical significance of data between different groups. If $P<0.05$, it was deemed significant. There were no outliers observed in the current study. In addition, no data normalization procedures were applied.

RESULTS AND DISCUSSION

The obtained results were exposed to one-way analysis of variance followed by *post hoc* Tukey's test to assess the statistical significance of data between different groups.

Antioxidant and Stress Biomarkers

Citalopram treatment resulted in significant ($p < 0.0001$) decrease in the activity of antioxidant enzymes (SOD, CAT and GPx) in the testicular tissues, in addition significant rise ($p<0.0001$) of MDA levels within testes when compared to control animals (G1). No significant changes ($p > 0.05$) in the activity of antioxidant enzymes and MDA levels with exception of significant increase of SOD ($p < 0.05$) were observed in treated groups with ginseng (G3) and vitamin D (G4) alone in comparison with control group. Co-administrations of citalopram with ginseng (G5), vitamin D (G6), ginseng +vitamin D (G7) ensued a significant ($p < 0.001$) upsurge and marked enhancement in testicular SOD, CAT and GPx activity, with significant ($p < 0.001$) reduction in MDA levels when compared with G2 (Figure 1).

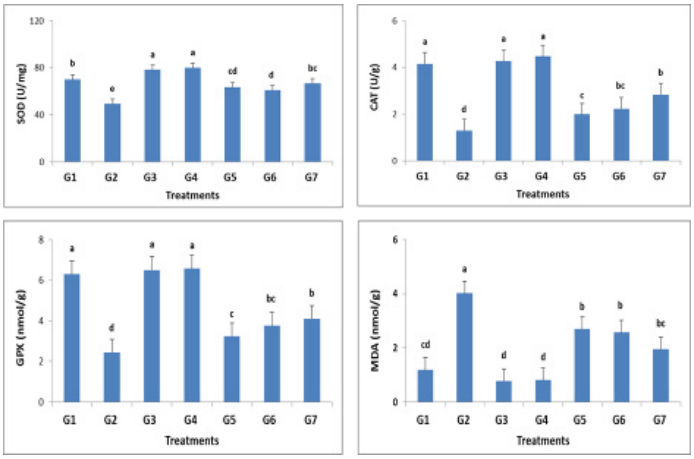

Figure 1: Effects of various treatments on antioxidant enzymes (SOD, CAT & GPx) and MDA levels in the testicular tissue of rats after 60 days of experiment. The different alphabetical letters (a- d) indicate a significant probability ($p < 0.05$).

Hormonal Analysis

Repeated oral treatment of citalopram (1.8 mg/kg/day) for 60 days resulted in significant ($p<0.001$) rises in the serum levels of LH, and FSH with non- significant ($p > 0.05$) difference in TS level when compared to the untreated control (G1). Treatments of rats with ginseng (18 mg/kg b.wt) or vitamin D (0.01mg/kg b.wt) alone caused non-significant ($p > 0.05$) alterations in serum TS, LH, and FSH levels when compared to the untreated control values, while serum TS was significantly ($p < 0.05$) decreased in vitamin D- treated group. Repeated oral treatments of rats with citalopram in combination with ginseng (G5),vitamin D (G6), or both (G7) for 60 days resulted in significant declines in the circulating serum TS ($p<0.01$), LH ($p<0.001$), and FSH ($p<0.001$) levels when matched with the positive control (citalopram group) neighboring to the normal values of negative control (Figure 2).

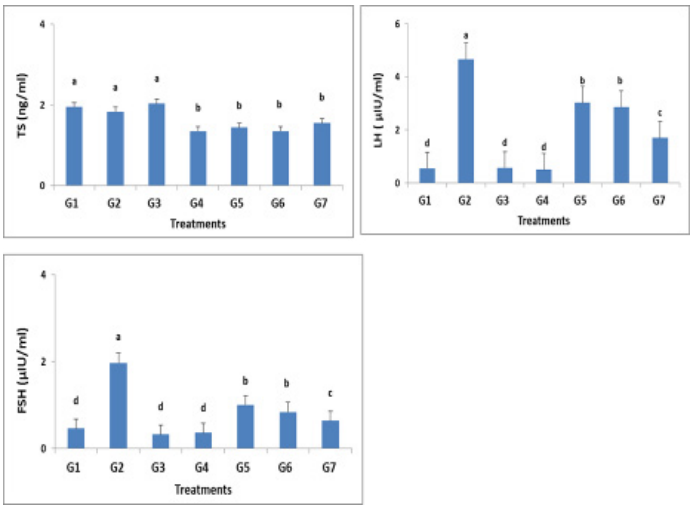

Figure 2: Effects of various treatments on serum testosterone (TS), luteinizing hormone (LH) and follicle -stimulating hormone (FSH) levels in rats after 60 days of experiment. The different alphabetical letters (a- d) indicate a significant probability ($p < 0.05$).

Semen Analysis

When compared to the control group, the citalopram-treated group showed a significant reduction ($p<0.001$) in sperm count and motility and a significant rise ($p<0.0001$) in the percentage of sperm abnormalities (Table 1). However, co-administration of citalopram with ginseng, vitamin D, and ginseng plus vitamin D to normal rats caused significant ($p< 0.0001$) improvement in count of sperms, motility and sperm morphology, compared with the positive control (G2). This improvement was towards the normal of G1. Treated rats with either ginseng or vitamin D alone showed nearly similar sperm parameters when compared with the negative control values (Table 1).

Histopathological Findings

Upon histological analysis of the testicular tissues of the control group (G1), seminiferous tubules (ST) were found to be seemingly normal. The lumen of the tubules was filled with spermatids, regular spermatogonia, and a sequence of spermatocytes. The interstitial tissue exhibited a variable number of Leydig cells. The seminal vesicles (SV) denoted

Table 1: Semen analysis in the control and treated groups of adult male rats after 60 days of experiment. Mean ± SEM (n= 6).

Parameters/ Groups	Sperm concentration ($x10^6$/ml)	Sperm motility (%)	% Abnormal sperms
G1 (-ve control)	107.67±2.60 ^{ab}	86.33±3.66 ^{ab}	15.67±0.51 ^{b}
G2 (Citalopram)	53.00±22.11 ^{b}	56.67±2.32 ^{c}	28.69±2.92 ^{a}
G3 (Ginseng)	156.00±8.66 ^{a}	93.33±2.85 ^{a}	11.97±0.77 ^{bc}
G4 (Vit D)	136.00±14.73 ^{a}	93.30±3.07 ^{a}	10.89±0.37 ^{bc}
G5 (Citalopram+ ginseng)	128.33±21.40 ^{a}	80.33±2.66 ^{b}	13.47±0.45 ^{bc}
G6 (Citalopram+ Vit D)	108.67±6.01 ^{ab}	81.32±3.00 ^{b}	14.15±0.14 ^{bc}
G7 (Citalopram+ ginseng +Vit D)	116.00±7.21 ^{a}	83.33±2.44 ^{b}	13.02±0.18 ^{bc}

Means in the same column not followed by the same letter differ significantly (p<0.05).

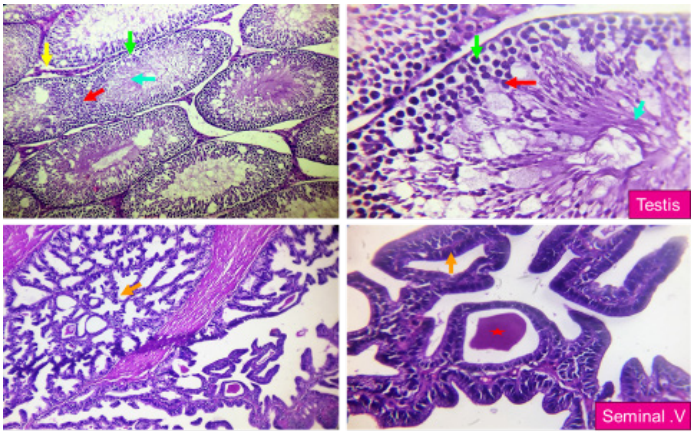

Figure 3: Photomicrographs from rat's testis and seminal vesicle of control free group showing apparently normal seminiferous tubules with regular normal spermatogonia (green arrows) and a series of spermatocytes (red arrows) followed by spermatids and normal spermatozoa which fill the lumen of the tubules (light blue arrows). The interstitial tissue showed a variable number of Leydig cells (yellow arrow). The seminal vesicle denotes a normal structural appearance with seminal acinar structures and papillary fronds lined by simple secretory columnar epithelium (orang arrows) and eosinophilic intra-luminal secretory materials (red asterisks). H&E X 200, 400

a normal structural appearance with seminal acinar structures and papillary fronds lined by simple secretory columnar epithelium and eosinophilic intra-luminal secretory materials. No pathological changes could be detected in any stage of examined testicular or SV tissues (Figure 3). Citalopram-treated group (G2) revealed moderate destruction of the ST affecting about 30 to 45% of the tubular structures including some of them in the central tubules and others at the periphery. The affected ST showed marked interstitial edema, vascular dilatation, edema, and degenerative and necrotic changes in the germinal epithelium, spermatocyte, and spermatozoa. Some of the ST showed degeneration of the primary and secondary spermatozoa with vacuolation of the spermatogonia. The interstitial tissue in some tubules appeared thickened by Leydig cells proliferation and edema. Other ST appeared atrophied with irregular contours. The SV thickened interstitial tissue. Other parts of the SV showed cystically dilated papillae and acini with large amounts of secretory materials, atrophy of the lining epithelium, and interstitial edema. The proportion of the normal to the cystically dilated parts is one-fifth (1:5) (Figure 4). Ginseng-treated group (G3) showed normal structure compared to the control group with keeping features of the regarding the germinal epithelium, spermatocyte, spermatids, and spermatozoa. The SV revealed normal structures including the acini and the papillary fronds with normal secretory materials and lining epithelium. Mild interstitial edema and fibroblastic proliferation could be detected in some lobules. A few lobules showed cystic degeneration (Figure 5). Vitamin D-treated group (G4) revealed highly active ST with the presence of dividing spermatocytes and well-formed spermatocytes and spermatozoa. However, focal thickening of the interstitial fibrous tissue and cystic dilatation of some follicles could also be detected (Figure 6). Citalopram plus ginseng-treated group (G5) revealed about 25-30% of the ST with characteristic cytotoxic effect most probably citalopram-induced as represented by marked necrosis and/or apoptosis of the affected ST cells. The interstitial tissue showed edema and atrophy of some tubules. The remaining testicular tissue showed actively normal tubular structure with apparently healthy ST with normal histo-morphological structures. The SV showed a focal cystic change in the acinar and papillary structures with partial atrophied epithelium calls. There was also interstitial edema and vascular dilatation. The remaining SV, follicles, and papillary structures were apparently normal (Figure 7). In citalopram plus vitamin D-treated group (G6), about 5-10 % of testicular ST showed degenerative and early necrotic changes in the cellular structures together with focal atrophy and disorganization of the tubules. Focal interstitial edema and vascular dilatation were also detected. The interstitial septa were mildly thickened due to edema, fibroblastic and Leydig cell proliferation. The SV showed mild to moderate interstitial edema and fibrosis besides cystic dilatation of some follicles. The remaining part showed a normal configuration of the papillary and follicular structures with apparently normal columnar epithelium cells and eosinophilic secretory materials (Figure 8). Citalopram combined with ginseng and vitamin D (G7) showing that about 5-10% of the testicular ST were affected as represented by mild to moderate degenerative, apoptotic and necrotic changes in tubular linings, focal interstitial edema with atrophy of some tubules could also be detected. The remaining testicular tissue showed normal ST structures with active spermiogenesis and spermato-

genesis. The Leydig cells appear with normal structure and normal population. However, a slight rise in the number of Leydig cells could be observed in some cases. Apparently normal seminal vesicular structures with keeping features of the folliculo-papillary pattern and active secretory function could be recorded. Mild interstitial fibrosis was seen in a few cases. The lining epithelium of the follicle and papillae appeared normal (Figure 9).

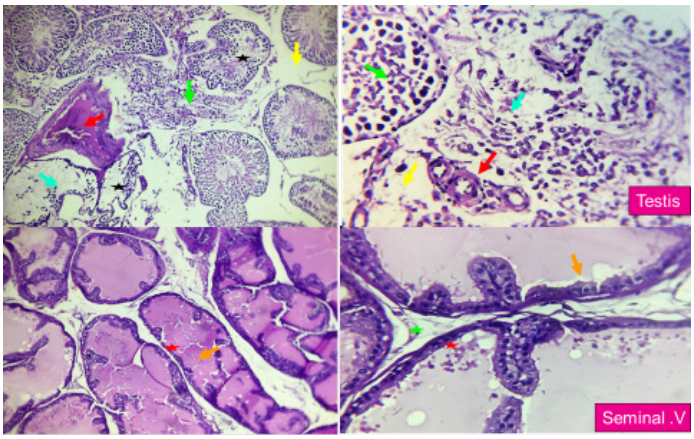

Figure 4: Photomicrographs from rat's testis and seminal vesicle of citalopram treated group (G2) showing moderate destruction of the seminiferous tubules with marked interstitial edema (yellow arrows), vascular dilatation (red arrows), and degenerative and necrotic changes in the germinal epithelium, spermatocyte, and spermatozoa (light blue and green arrows). Other seminiferous tubules appeared atrophied with irregular contours (black asterisks). The seminal vesicle shows cystically dilated papillae and acini with large amounts of secretory materials, (orange arrows) atrophy of the lining epithelium (red asterisks), and interstitial edema (green asterisk). H&E X 100, 400

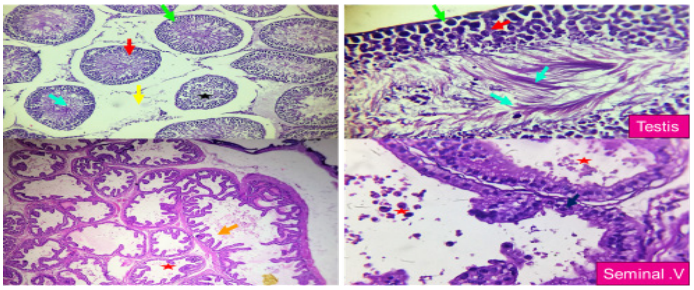

Figure 5: Photomicrographs from rat's testis and seminal vesicle of ginseng treated group (G3) showing focal mild interstitial edema (yellow arrow) with partial atrophy of some seminiferous tubules (black asterisk), otherwise, most of the testicular tissue appears normal with keeping features of the seminiferous tubules regarding the germinal epithelium (green arrows), spermatocyte (red arrows), spermatids, and spermatozoa (light blue arrows). Seminal vesicle revealed normal structures including the acini and the papillary fronds (orange arrow) with normal secretory materials (red asterisks) and lining epithelium (dark blue arrow). H&E X 100, 400.

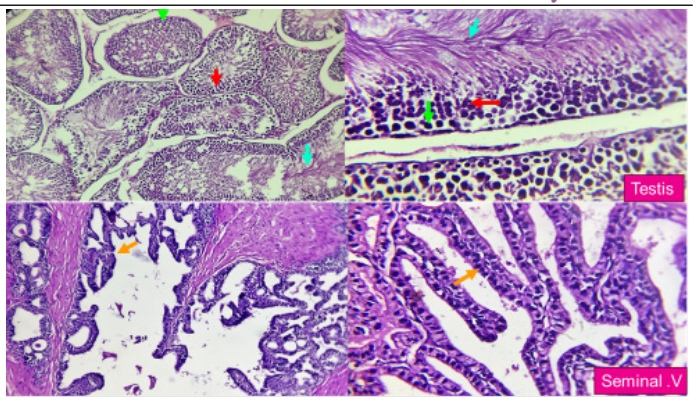

Figure 6: Photomicrographs from rat's testis and seminal vesicle of Vit. D treated group (G4) showing active seminiferous tubules with the presence of dividing spermatocytes and well-formed spermatocytes and spermatozoa (green, red and light blue arrows). The seminal vesicle showed normal configuration regarding the acini and the papillary structure (orange arrows). H&E X 200, 400

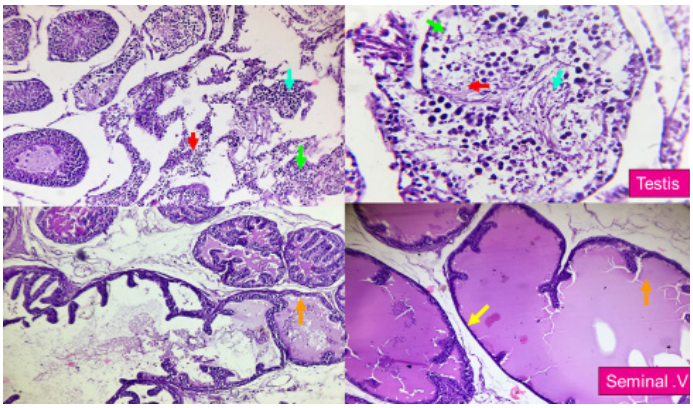

Figure 7: Photomicrographs from rat's testis and seminal vesicle of citalopram – Ginseng treated group (G5) showing about 25-30% of the seminiferous tubules with characteristic cytotoxic effect as represented by marked necrosis and/or apoptosis of the affected seminiferous tubular cells (green, red and light blue arrows). The seminal vesicles shows focal cystic change in the acinar and papillary structures (orange arrows) with partial atrophy of the epithelium lining (yellow arrow). H&E X 100, 400.

One hypothesis for the etiology of male infertility is oxidative stress (Kumar *et al*., 2013). Studies have publicized an augmentation in the levels of ROS in 40 to 80% of infertile men (Hamada *et al*., 2012). An imbalance between oxidants and antioxidants, favoring oxidants, can lead to oxidative stress, which can deteriorate testicular structure and spermatogenesis processes, ultimately resulting in infertility (Guz *et al*., 2013). Cells have antioxidant enzymes such as glutathione reductase (GR), SOD, CAT, GPx, and GPx to guard against the potentially detrimental effects of ROS. These enzymes mitigate the destructive effects of ROS by inactivating them. Antioxidant enzymes therefore serve as the cell's defense mechanism (Nita and Grzybowski, 2016).

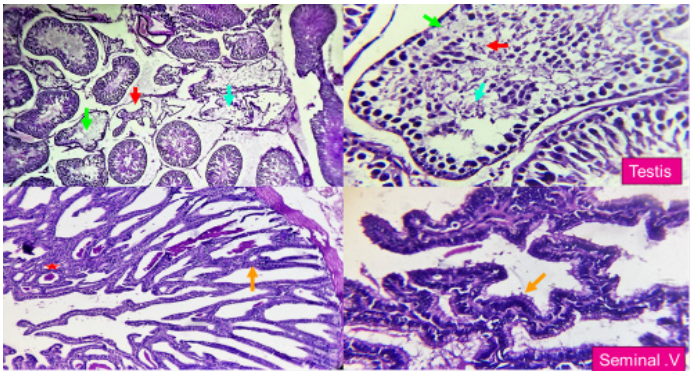

Figure 8: Photomicrographs from rat's testis and seminal vesicle of citalopram - Vit.D treated group (G6) showing about 5-10% of the seminiferous tubules with degenerative and early necrotic changes in all the cellular structures together with focal atrophy and disorganization of the tubules (green, red and light blue arrows). The seminal vesicles denote. Normal configuration of the papillary and follicular structures with apparently normal columnar epithelium cells (orange arrows), and eosinophilic secretory materials (red asterisk). H&E X 100, 400

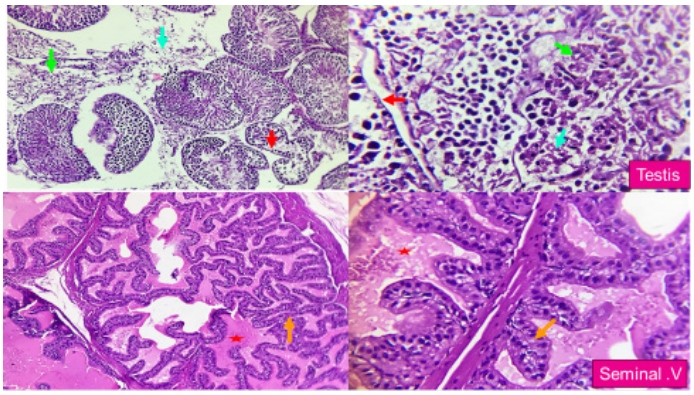

Figure 9: Photomicrographs from rat's testis and seminal vesicle of citalopram cojoined with Ginseng and Vit.D (G 7), showing about 5-10% of the seminiferous tubules with mild to moderate degenerative, apoptotic and necrotic changes in seminiferous tubules (green, red and light blue arrows). The seminal vesicles shows normal seminal vesicular structures with keeping features of the folliculo-papillary pattern (orange arrows) and active secretory function (red asterisks). H&E X 200, 400

The results of current study showed significant decrease in SOD, CAT and GPx activities with increased levels of MDA in testicular tissue of citalopram-treated rats. This is considered as indicator of deterioration in the antioxidant / oxidant balance in the citalopram-administered group. The present findings are consistent with previous studies (Hashim, 2020). Marked alterations noticed in antioxidant activities in the testis of citalopram-treated rats might have been initiating and propagating the oxidative damage (Kumar *et al*., 2024). Spermatogenesis is hampered by lipid peroxidation (LPO), which breaks down the lipid matrix's structure in the membranes of germ cells and spermatozoa. Testicular MDA activity is increased, which is indicative of damaged cell membranes caused by high LPO levels (Herbet *et al*., 2015). In conventionality with prior investigations (Atli *et al*., 2017), administration of sertraline initiated a state of oxidative stress in the rat testicular tissue, revealed by augmented lipid peroxidation and cooperated antioxidant defense system. Recent evidence stated that fluoxetine as a one of SSRI increased MDA level and decreased the antioxidant enzyme activity (SOD, CAT and GSH) (El Sayed *et al*., 2021). Furthermore, citalopram administration significantly increased oxidative stress with significantly increased MDA levels (Moradi *et al*., 2023). Administration of ginseng and vitamin D in combination with citalopram caused an improvement in the antioxidants and MDA levels. This improvement in the antioxidant enzymes and amelioration in MDA levels improved infertility in aged rats treated with ginseng (Won *et al*., 2014) or vitamin D (Maghsoumi-Norouzabad *et al*., 2021).

Endocrine activity, which is mediated by the hypothalamic-pituitary-testes axis, controls spermatogenesis (Ciarrocca et al., 2013). FSH and LH are secreted by the anterior pituitary, and testosterone is secreted by the testes to carry out crucial functions in spermatogenesis. LH promotes the production of testosterone from Leydig cells in the testes, while FSH promotes the production of androgen-binding proteins from Sertoli cells during the process of sperm maturation. Testosterone is essential for the maintenance of semen production and the spermatogenesis process (Wdowiak *et al*., 2014).

Citalopram treatment for 60 days increased the sera LH, and FSH levels with non-significant ($p > 0.05$) difference in serum TS level. The hormonal results of the present study were in agreement with other reported investigations (Erdemir et al., 2014). There were preceding reports showed some differences in the evaluations of hormonal parameters. Sertraline administration resulted in a significant increase in serum TS (Atli *et al*., 2017), increased FSH and lower TS levels with no significant differences in the LH levels (Hamdi, 2019). Moreover, a repeated oral treatment citalopram (10 mg/kg) twice daily for 21 consecutive days significantly increase serum TS concentration (Przegaliński *et al*., 1987). When rats were administered citalopram orally once daily at varied concentrations for four weeks in a row, the study did not uncover a significant variation in their testosterone levels (Khan *et al*., 2023). In addition, adult male rats given 200 mg/kg of fluoxetine for 60 days demonstrated a noteworthy drop in TS and FSH levels (Bataineh and Daradka, 2007). Testicular failure is known to be indicated by elevated LH and FSH levels with normal TS levels (Ilgin *et al*., 2017).

Oral treatment of rats with citalopram in combination with ginseng, vitamin D, or both resulted in significant declines in the circulating serum TS ($p<0.01$), LH ($p<0.001$), and FSH

(p<0.001) levels when matched with citalopram group. These results indicated that an improvement in the hormones levels particularly LH and FSH and the potential benefits of the used ginseng or vitamin D against testicular damage in rats induced by citalopram. Ginseng therapy is crucial for preserving essential sex hormone receptor levels, which in turn promotes healthy androgen production and regular spermatogenesis (Lee *et al*., 2019). Vitamin D treatment decreased the adverse effects of citalopram causing infertility. An investigation showed that the TS and LH concentrations were significantly decreased during vitamin D treatment (Behmanesh *et al*., 2019).

With reference to semen analysis in the existing study, citalopram treatment for 60 days lowered sperm concentration, motility, and normal sperm morphology with increases in the percentage of sperm abnormalities. Reduction in sperm count and motility may be attributed to oxidative stress made by high production of free radicals as a result of testicular damage-induced by citalopram. Sperm cell membranes are rich in polyunsaturated fatty acids, making them the target of the ROS produced. (Dandekar *et al*., 2002). The consumption of mitochondrial oxygen and the production of ROS are recognized to elevate during the fertilization and spermatogenesis process (Guerriero *et al*., 2014). An excess of ROS can result in infertility due to a reduction in sperm count and motility (Kumar *et al*., 2013). Abnormal sperm morphology is produced during the process of sperm membrane remodeling in spermatogenesis, along with the generation of ROS. (Guerriero *et al*., 2014). Serum hormone levels, specifically FSH and LH, were negatively correlated with sperm concentration, motility, and morphology (Keskin *et al*., 2015). Other papers that demonstrate a decrease in normal sperm morphology, concentration, and motility after citalopram exposure corroborated our findings (Elnazer and Baldwin, 2014). The increases in morphologically abnormal sperm percentage (28.69±2.92%) in this study are considered as an important indicator of disorders that occur during sperm production and maturation that lead to infertility. Male infertility is generally regarded to be present when sperm abnormalities are found to be more than 10%. (Saba *et al*., 2009). Morphological abnormalities in the sperm alone are thought to influence the sperm's fertilization potential (Agarwal *et al*., 2014). Similarly, the sperm concentration and normal sperm morphology were significantly decreased in rats received oral doses of citalopram hydrobromide at 5, 10, and 20 mg/kg/day for 28 days (Ilgin *et al*., 2017). The percentage of sperm count and sperm motility were significantly decreased while the sperm abnormalities was increased with increased dose of antidepressant drug fluoxetine treatment (2.6, 7.8 and 13.0 mg/kg b.wt.) for 5 consecutive days (Alzahrani, 2012). Likely, Atli *et al*. (2017) showed that sertraline decreased the sperm concentration, motility, and increased abnormalities of sperm morphology, induced histopathological changes in testicular tissue.

The quality of the semen was enhanced by ginseng and/or vitamin D treatment. Our results align with previous study, since multiple preclinical studies have demonstrated that ginseng, as a cyclic adenosine monophosphate-responsive element modulator, enhances spermatogenesis and improves testicular issues, sperm quality, and sperm motility (Lee *et al*., 2020). Ginseng has been shown to increase sperm count and quality in both healthy persons and treatment-related infertile patients (Leung and Wong, 2013). As well as other studies recorded the benefits effect of vitamin D on spermatogenesis and sperm parameters (BaSalamah *et al*., 2018). Comprehensive studies confirmed that supplementing with fluoxetine and vitamin D significantly decreased testicular damage and restored all pertinent parameters (El Sayed *et al*., 2021).

Histopathological examinations are often used as important biomarkers in toxicological researches. Histopathological changes in the testes can accompany decreases in sperm quantity and quality (Ben Slima *et al*., 2017). In the current study, citalopram induced injurious effects on the testicular tissue including marked interstitial edema, vascular dilatation, edema, and degenerative and necrotic changes in the germinal epithelium, spermatocyte, and spermatozoa in ST matched to the control group. The seminal vesicle showed cystically dilated papillae and acini with large amounts of secretory materials, atrophy of the lining epithelium, and interstitial edema. The histopathological observations confirmed that citalopram caused testicular damage. These findings are in harmony to previous reporters (Elsedawi *et al*., 2021; Moradi *et al*., 2024). Such findings observed in the current study were in harmony with others in which SSRIs use caused significant damage to the testicular histology (Soliman *et al*., 2017). Other researchers (Ilgin *et al*., 2017) revealed sever testicular damage including germinal cell degeneration, atrophy of STs, extracellular vacuolization, and reduction of cells in the spermatogenesis series, especially at high doses of citalopram. Furthermore, fluoxetine treatment resulted in marked alterations in the testis, indicated by disorganized and shrunken STs, thickening of the tunica propria, and vacuolization of germ cells (Kumar *et al*., 2024), cells with darkly stained nuclei (denoting apoptosis) and separated epithelium from the underlying basement membrane, degenerated germ cells were detached and found in the lumen of the tubules (Elsedawi *et al*., 2021), In addition to deformation of the seminiferous tubules, deteriorated spermatogenic cells with extensive cytoplasmic vacuoles, and sloughing of germ cells. (Hajizadeh *et al*., 2016; Sayed *et al*., 2021). The aforementioned findings noticed in the existing work in which citalopram usage initiated major damage to the testicular histology which was upturned in the treated rats (G5-7). In comparison to the citalopram group, the his-

topathological lesions in the testis and SV were at a lower level in the citalopram- treated rats with ginseng (G5), vitamin D (G6), or both (G7). The affections of STs were mostly better in rats of groups 6 and 7 (about 5-10%) thatgroup5 (about 25-30%). The existing investigations verified that vitamin D or with ginseng can provide promising protection against citalopram -induced testicular damage. These results may be attributed to ginsenosides, and saponins which are the foremost active components of ginseng (Li *et al*., 2014), or due to vitamin D metabolizing and signaling molecules have all been identified in male and female reproductive organs from different species (Carlberg and Campbell, 2013; Mohamed *et al*., 2021). These findings go in agreement with previous studies (Kopalli et al., 2015; Sayed *et al*., 2021; Moradi *et al*., 2024). Earlier studies showed that ginseng protected against testicular damage induced by doxorubicin in rats (Chen et al., 2013), while vitamin D ameliorated testis injury caused by imidacloprid (Keles *et al*., 2022), or lead (BaSalamah *et al*., 2018).

Treatments with ginseng and vitamin D alone produced no histopathological changes in the testicular and SV architecture, STs, and spermatozoa (spermatogenesis) when compared to the control group. Similar results were reported in former models for ginseng (Shojaeepour *et al*., 2022) or vitamin D (Marahovskiy *et al*., 2022).

CONCLUSIONS AND RECOMMENDATIONS

The results of the current investigation indicated that ginseng and vitamin D could improve the citalopram-induced damage of testicular tissue and impaired sexual function. Such effects might be attributed to their pronounced antioxidant activities. Therefore, ginseng and vitamin D might act as potential candidates to improve citalopram-induced testicular toxicity and damage in male rats.

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NOVELTY STATEMENT

This study indicated the importance of ginseng and vitamin D to improve citalopram-induced male infertility in rats

AUTHORS' CONTRIBUTIONS

All authors directly contributed to the manuscript's composing, revising, and approving its final version. Gamal E. Shams, Gihan G. El-Sayed, Reda M. Abd El-Aziz developed the study design and controlled its progress. Aya Salah Eldin Mohamed, and Reda M. Abd El-Aziz performed the animal treatment, examination, and follow-up. Aya Salah Eldin Mohamed, Gamal E. Shams, and Gihan G. El-Sayed analyzed the estimated parameters obtained throughout the study. Aya Salah Eldin Mohamed performed the statistical processing of the experimental data.

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Conflict of Interest

None of the authors have any conflict of interest to declare.

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